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THE USE OF NON-UNIFORM ELECTRIC FIELD FOR LIVE/DEAD DETERMINATION OF ZOO- AND ICHTHYOPLANKTON

Report No. 84-196-K

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ABSTRACT

Use of a non-uniform electric field was evaluated as a method to expedite the separation of living and dead plankters. Low frequency sine waves at the highest non-lethal voltages were most successful. This method was found to be 98% effective for separating live and dead Daphnia magna and 79% effective for separating round whitefish, Prosopium cylindraceum eleutheroembryos.

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To Mr. F.J. Kee
Director of Research

The use of a Non-Uniform Electric Field
for Live/Dead Determination of Zoo- and Ichthyoplankton

INTRODUCTION

The effect of entrainment on plankton has been examined in terms of numbers passing through the generating station and the mortality suffered during condenser passage. Existing studies have concentrated on the crustacean zooplankton, with mortality being estimated by direct sorting, using motility to distinguish living zooplankton from the dead (OHRD Reports 77-225-K,78-208-K). This method is time consuming and tedious and can place considerable restraints on sample size.

Other more effective methods such as vital and mortal staining techniques have been used to expedite the live/dead determinations (Dressel et al 1972, Crippen and Perrier,1974, OHRD Report 78-469-K). These techniques however, are subject to the complications of stain induced mortality and variable stain intensity.

The use of a divergent electric field to distinguish live/dead plankton may overcome the shortcomings of the above techniques. The objective of this study was to evaluate the use of a non-uniform electric field for live/dead determinations of a representative zoo- and ichthyoplankter.

METHODS AND MATERIALS

a) Theory

Any neutral object will have a finite separation of equal amounts of positive and negative charges (Pohl and Hawk, 1966). Placed between two parallel but oppositely charged electrodes (uniform electric field) it will experience an equal but opposite pull from each electrode (Figure 1). The net result is the neutral object will remain stationary. If the object is placed in a non-uniform (or divergent) electric field alignment of an equal number of positive and negative charges to their opposite charged electrode will occur, however, one end of the object will be in a weaker field than the other. Thus a net force will result and the object will be pulled towards the region of greatest field intensity. This phenomenon has long

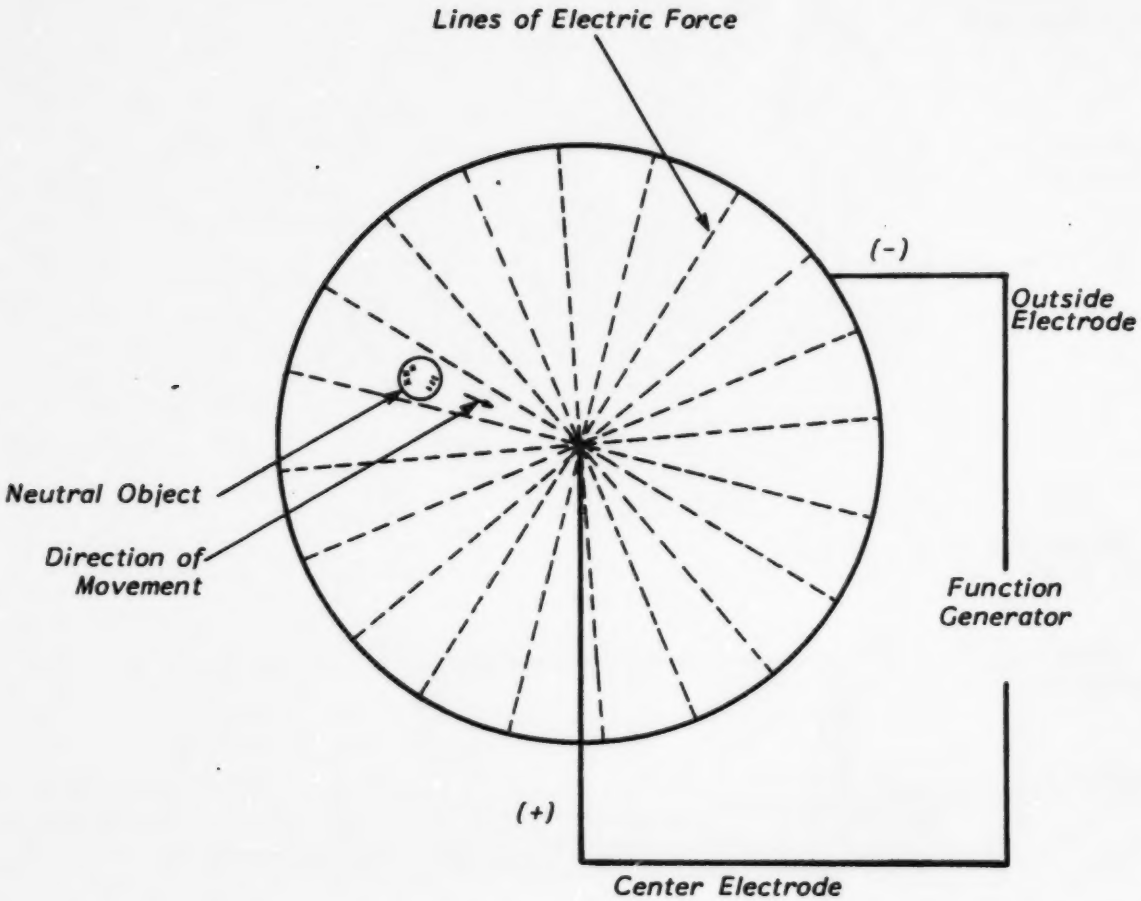


FIGURE 1

ILLUSTRATION OF THE NON-UNIFORM FIELD WHICH WILL EXIST BETWEEN OPPOSITELY CHARGED CYLINDRICAL ELECTRODES. A NEUTRAL OBJECT WILL MOVE TOWARDS THE CENTER ELECTRODE OR THE AREA OF GREATEST FIELD INTENSITY

been recognized and is known as dielectrophoresis (Pohl 1960). Dielectrophoresis is distinct from electrophoresis in that the latter is the motion of charged particles in an electric field. Their direction of movement is dependent upon their respective charge. Dielectrophoretic movement is only dependent upon differences in field intensities.

The success of the dielectrophoretic process is dependent upon the existence of a difference in the polarizability between the object and medium. This difference is a function of the dielectric constant and the specific conductivity of the object and medium. Since a significant difference appears to exist between water and living tissue (Schwan 1957) it may be possible to separate live/dead organisms.

b) Experimental Apparatus

The dielectrophoresis cell was constructed of 'Perma-Base' denture acrylic, with embedded stainless steel electrodes. The cell was circular in shape with a cup-shaped cavity (Figure 2). The diameter of the cell was 55 mm. The depth ranged from 8 mm at the outside electrode to 12 mm at the center electrode. The stainless steel electrodes were connected to a high voltage function generator capable of producing three wave forms (sine, square, and triangular), 255 volts peak to peak and a variable frequency (0 - 7623 Hz).

c) Test Species

Laboratory reared adult Daphnia magna were used as the representative zooplankter. Fish species were represented by embryos and eleutheroembryos of round whitefish (Prosopium cylindraceum).

d) Experimental Design

Replicate samples of live D. magna (25 individuals) and round whitefish embryos and eleutheroembryos (20 of each) were used to assess the attraction efficiency of the center electrode. Movement of organisms to within 20 mm of the central electrode was considered to be demonstrative of attraction. This area accounted for only 15% of the dielectrophoresis cell. During initial tests to determine which combination of electrical parameters resulted in the greatest attraction, only D. magna were used. During these tests the zooplankers were placed in dechlorinated tap water while voltage, frequency and wave form were varied. These tests were conducted for three wave forms (sine, square, and triangular), over a range of 10 to 40 volts, and at frequencies from 0 to 7623 Hz.

Subsequent tests designed to improve the attraction involved varying the medium in the cell using deionized and distilled water, and the duration of non-uniform field conditions from 1 to 3 minutes. At the end of each treatment the number of D. magna within 20 mm of the center electrode were counted and

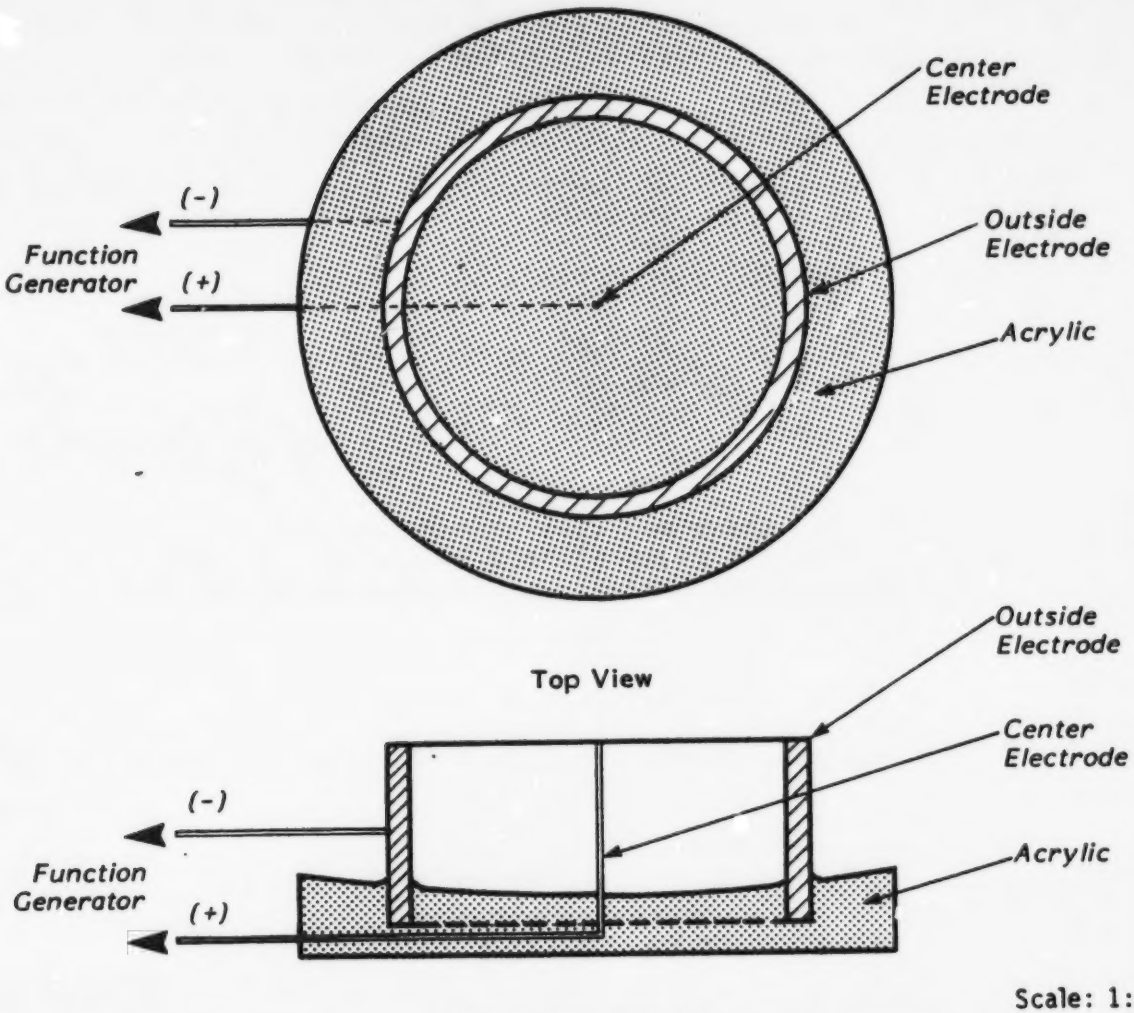


FIGURE 2

ILLUSTRATION OF THE DIELECTROPHORETIC CELL USED TO DETERMINE THE INFLUENCE OF A NON-UNIFORM ELECTRIC FIELD ON LIVE Z00 AND ICHTHYOPLANKTERS

expressed as a percent of the total. Percent mortality following treatment was also noted. Electrical parameters which resulted in the greatest attraction of D. magna were used during the live/dead determinations for D. magna and round whitefish embryos and eleutheroembryos.

Live/dead separation was evaluated with premixed single blind combinations of live and dead organisms, distributed randomly throughout the cell. The dead organisms were killed by exposure to 50°C water for one minute. Live/dead determinations for both D. magna and whitefish were replicated ten times. Prior to application of the electric field the number of non-motile organisms within the 20 mm circle was noted and these were considered dead. During application of the electric field, organisms within 20 mm of the center electrode were counted a second time. The number of live organisms was estimated as the second count minus the initial dead. Separation efficiency was determined by expressing the difference between actual and estimated live/dead as a percentage for both D. magna and round whitefish.

Results and Discussion

Application of a non-uniform electric field resulted in both living adult Daphnia magna and round whitefish eleutheroembryos being involuntarily attracted to the center electrode. Movement to the center electrode was completed in less than 30 seconds.

Three way analysis of variance was used to separate the effects of wave form, voltage, and frequency (Table 1a) for live Daphnia magna. The parameters wave form and voltage, and their interaction were responsible for the strongest effects. Greatest attraction (67%) was observed for sine wave at the highest voltage level (Figure 3). Over the range of frequencies examined, generally frequencies below 3000 Hz resulted in greatest attraction (Table 1b).

Subsequent tests designed to improve the attraction of live Daphnia involved increasing the applied voltage from 40 to 50 volts, and varying the duration from 1 to 3 minutes, as well as changing the electrolyte (medium) to either deionized or distilled water. Overall, attraction was improved to greater than 80% with either deionized or distilled water (Figure 4), however no significant difference was apparent between these two electrolytes (Table 2a). Increased durations of from 1 to 3 minutes showed no significant effect upon attraction. Voltage, remained a significant factor ($F_{[2,36]}=5.92$, $p<.05$), with greatest attraction observed at 40 and 50 volts, however attractions at 40 and 50 volts were not significantly different from each other (Table 2b). Treatment mortality (24 h) for Daphnia was less than 5% for field application times of one minute or less. Mortality following a three minute application was 100%.

Table 1a
Three Way Analysis of Variance
on Per Cent Live *Daphnia magna* Attracted
in Dechlorinated Tap Water

Source	Degrees of Freedom	Sums of Squares	Mean Square	F Ratio
Wave Form	2	7866.72	3933.36	18.90*
Voltage	2	49836.70	24918.35	119.75*
Frequency	9	6212.00	690.22	3.32*
FormXVolt	4	33944.54	8486.13	40.78*
FormXFreq	18	5173.39	287.41	1.38
VoltXFreq	18	3078.95	171.05	0.82
FormXVoltXFreq	36	4753.65	132.05	0.63
Within	180	37454.24	208.08	

* significant $p<0.05$

Table 1b
Multiple Range Tests Comparing
Mean Per Cent Live *Daphnia magna* Attracted
for The Factors Wave Form, Voltage and Frequency

Wave Form

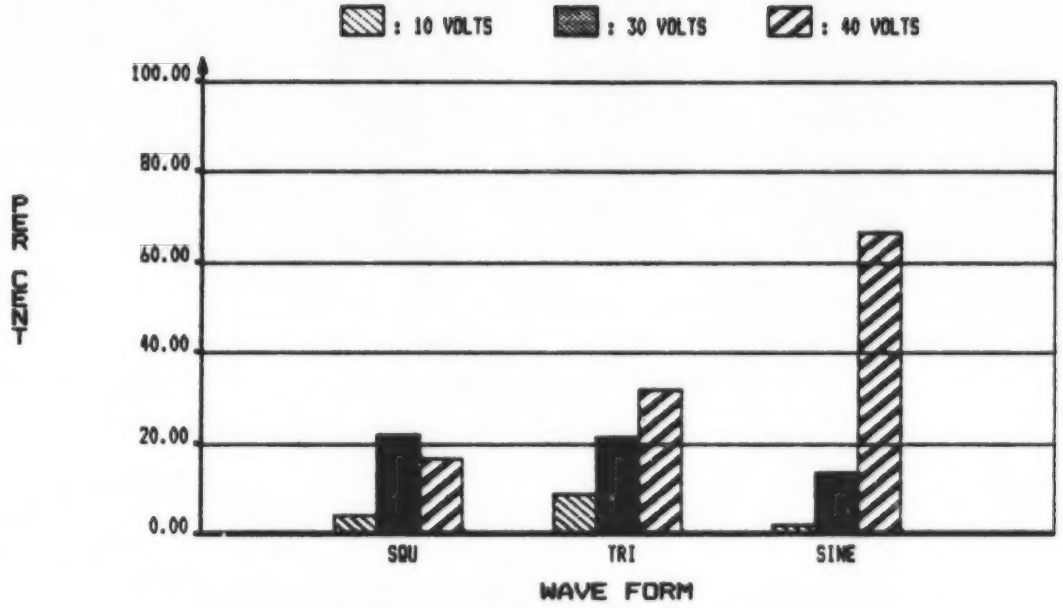
	Square	Triangular	Sine
Mean Per Cent	14.2	20.7	27.4
Grouping			

Voltage

	10	30	40
Mean Per Cent	5.2	18.8	38.3
Grouping of Samples			

Frequency (Hertz)

	7623	5980	6839	5137	2600	4285	36	1700	940	137
Mean Per Cent	15.9	16.7	17.2	17.8	19.0	19.6	20.6	21.1	29.7	29.9
Grouping of Samples										



SQU = SQUARE WAVE
TRI = TRIANGULAR WAVE
SINE = SINE WAVE

FIGURE 3
MEAN PER CENT LIVE DAPHNIA MAGNA
ATTRACTED TO WITHIN 20 MM OF THE CENTER
ELECTRODE AT 36 HZ IN THE EXPERIMENTAL CHAMBER
FILLED WITH DECHLORINATED TAP WATER

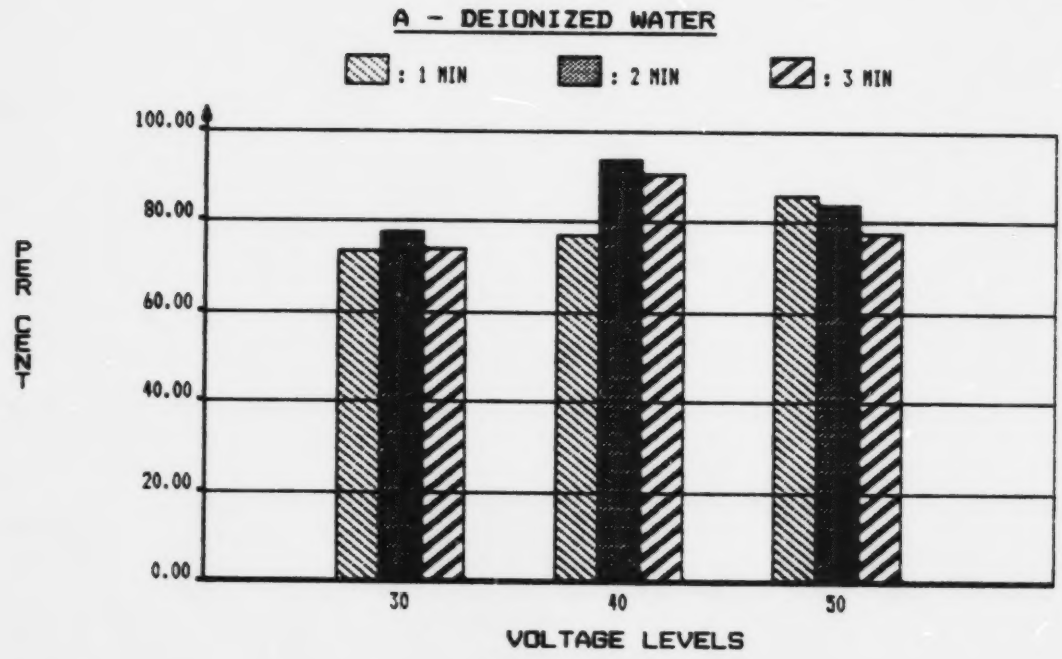
Table 2a
Three Way Analysis of Variance
on Per Cent Live *Daphnia magna* Attracted
For Combinations of Electrolyte, Voltage and Time

Source	Degrees of Freedom	Sums of Squares	Mean Square	F Ratio
Time	2	135.72	67.86	0.82
Electrolyte	1	21.16	21.16	0.26
Voltage	2	980.81	490.40	5.92*
TimeXEle	2	97.24	48.62	0.59
TimeXVolt	4	206.70	51.68	0.62
EleXVolt	2	410.43	205.21	2.48
TimeXEleXVolt	4	437.93	109.48	1.32
Within	36	2980.74	82.80	
Total	53	5270.73		

* significant $p<0.05$

Table 2b
Multiple Range Tests Comparing
Mean Per Cent Live *Daphnia magna*
Attracted for Three Voltages

Voltage	30 Volts	40 Volts	50 Volts
Mean Per Cent Attracted	75.0	82.9	84.9
Grouping of Samples	⌊——⌋	⌊————————⌋	



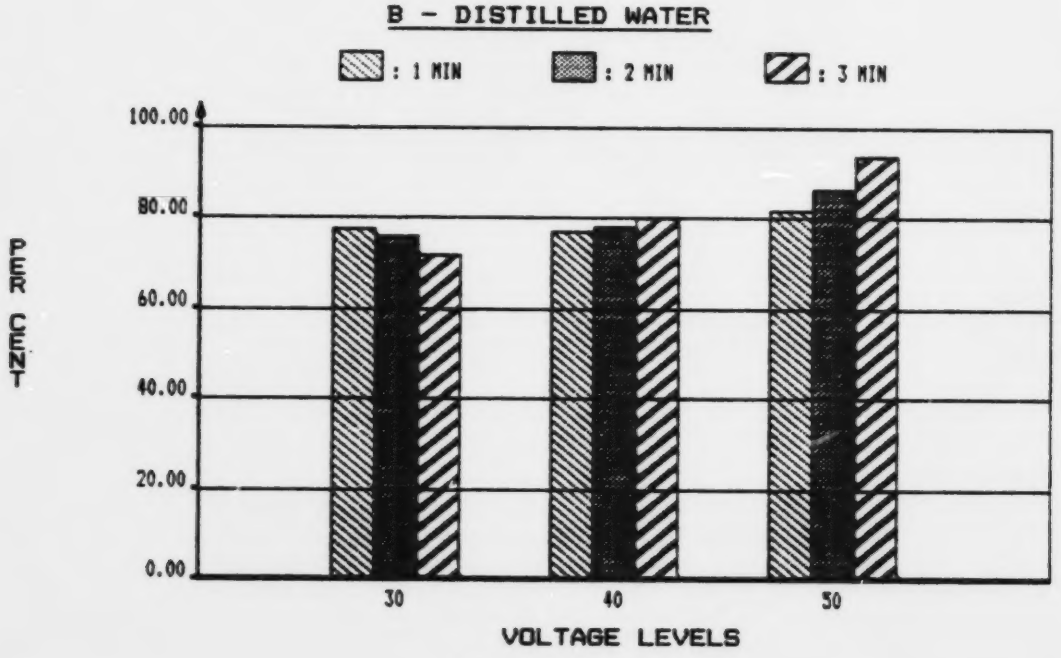


FIGURE 4
MEAN PER CENT LIVE *DAPHNIA MAGNA* ATTRACTED TO WITHIN 20 MM OF THE CENTER ELECTRODE USING SINE WAVE AND EXPERIMENTAL COMBINATIONS OF ELECTROLYTE AND VOLTAGE FOR THREE MINUTES DURATION AT 36 HZ

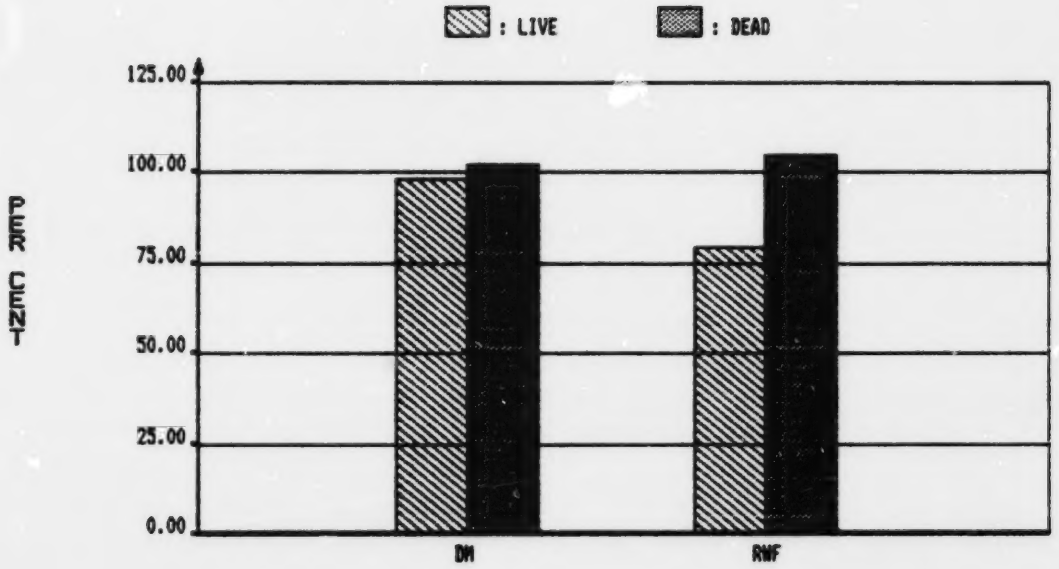
Round whitefish eleutheroembryos were first tested under the same conditions which had resulted in the greatest attraction of live Daphnia. These conditions resulted in complete mortality of the round whitefish eleutheroembryos. Size among fish has been directly correlated with sensitivity to an electric field (Northrop 1967). The size difference between Daphnia and round whitefish eleutheroembryos (13 mm), probably accounted for the mortality among eleutheroembryos. Subsequently further tests involving eleutheroembryos were conducted at a lower voltage (10 volts) using dechlorinated tap water, in order to reduce their sensitivity. These tests resulted in the attraction of 96% ± 5.8 SD of the live eleutheroembryos to the center electrode. Mortality (24 h) following treatment was less than ten percent.

Live round whitefish eyed embryos were tested under the same conditions found satisfactory for eleutheroembryos. Attraction of embryos to the central electrode did not occur. The embryos however, were observed to respond to the applied field by whole body movements within the egg.

The efficiency of separating live and dead organisms was tested using the combination of electrical parameters which resulted in the best attraction of live Daphnia and round whitefish. Tests involving D. magna or round whitefish eleutheroembryos, resulted in the live plankters congregating at the center electrode within 20 to 30 seconds after application of non-uniform field conditions, with the dead remaining stationary. The percent accuracy for estimating live and dead D. magna was 98.4 ± 3.5 SD and 102.3 ± 11.4 SD respectively. The percent accuracy for estimating live and dead round whitefish eleutheroembryos was 79.2 ± 24.7 and 104.7± 16.4 respectively (Figure 5). Over estimation of the dead for both plankters probably resulted from mortality of a few individuals during the application of current. Estimation of the live eleutheroembryos was influenced by dead individuals within the counting cell and is considered an underestimate. During non-uniform field conditions movement of live individuals was impeded and/or blocked by dead individuals. Live individuals blocked outside the 20 mm radius of the center electrode therefore would not have been counted.

Separation of live/dead individuals of a species by dielectrophoresis has previously been demonstrated for yeast cells (Pohl and Hawk 1966). Key factors involved in the separation were similar to those used for the separation of live/dead Daphnia with the exception of frequency. Our results do not confirm their theoretical expectation that the successful use of dielectrophoresis depends upon using quite high frequencies.

In summary, dielectrophoresis appears to be an efficient technique for the separation of live/dead zoo- and ichthyoplankton. The key parameters in the separation of live/dead plankters appear to be (i) low frequency, sinusoidal alternating fields (ii) non-uniform electric field and (iii) media of low conductivity. Application of non-uniform field



DM = Daphnia magna
RWF = Round whitefish eleutheroembryos

FIGURE 5
PER CENT OF ACTUAL DAPHNIA MAGNA
AND ROUND WHITEFISH ELEUTHEROEMBRYOS
ATTRACTED TO WITHIN 20 MM OF THE CENTER ELECTRODE

conditions for a duration of 1 minute resulted in maximum attraction to the center electrode. Durations exceeding 1 minute resulted in increased treatment morality (24h). Employment of dielectrophoresis should be preceded by an evaluation of the required electrical parameters for the size range of plankters in any given study. Separation of live/dead ichthyoplankton of greater than or equal to the round whitefish (14 mm) should be conducted in deeper cells so that dead in the cell cannot block the involuntary movement of the live to the center electrode.

Conclusions

1) Dielectrophoresis resulted in the involuntary attraction of live plankters to an area of greatest field intensity. Live estimates for Daphnia magna and round whitefish eleutheroembryos were 98.4% ± 3.5 and 79.2 ± 24.7 respectively, of the actual live. Round whitefish embryos were not attracted.

2) Wave form, voltage, frequency, and electrolyte all affected the strength of attraction. Greatest attraction was observed for the sine wave form at low frequencies when applied at highest non-lethal voltages (50 v).

Approved:

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